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14. ABSTRACT

We have developed a novel strategy that combines tumor immunotherapy targeting PAP and targeted immune modulation of CTLA4 and have generated a lead cellular therapy that will safely enhance vaccine-mediated immunity. This lead cellular therapy, called DC-PAPvac-C, consists of dendritic cells (DCs) co-transfected with prostate tumor antigen, PAP RNA and anti-CTLA4 RNA. In this study we will establish the preclinical efficacy and safety of our cellular therapy product, DCs transfected with RNA that encodes PAP and anti-CTLA4 and generate data required for an Investigational New Drug (IND) application. Importantly and relevant to our planned clinical trial implementation, we will develop a biomarker of therapeutic efficacy and demonstrate the feasibility of measuring these biomarkers. In this report we have demonstrated that local CTLA4 modulation in combination with PAP-specific immunization using RNA-transfected DCs elicits robust and superior functional T cell responses in TRAMP mice. Using human cells, we have performed FDA mandated validation of DC-PAPvac-C to confirm anti-CTLA4 mAb expression and PAP presentation by human DCs transfected with mRNA encoding human PAP and anti-human CTLA4. Finally, we have completed the three cGMP production runs including lot release testing of the final DC-PAPvac-C cellular vaccine product.

15. SUBJECT TERMS
dendritic cell vaccine, dendritic cells electroporated with RNA, immune checkpoint blockade, local CTLA-4 modulation, prostate cancer immunotherapy, prostatic acid phosphatase (PAP), RNA-based vaccines

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Novel immune modulating cellular vaccine for prostate cancer immunotherapy (PC121288)
Principal Investigator: Smita Nair, PhD

INTRODUCTION:

The goal of immunotherapy is to stimulate T cells that recognize and destroy tumor cells; however, a major challenge to greater vaccine efficacy is immune suppression mediated by inhibitory receptors on activated T cells, specifically cytotoxic T lymphocyte antigen 4 (CTLA4). Systemic administration of anti-CTLA4 blocking antibodies has demonstrated clinical effectiveness in melanoma patients, but, consistent with its mode of action, anti-CTLA4 antibody causes significant immune-related adverse events. Strategies for delivering anti-CTLA4 to the site of T cell activation while limiting systemic exposure are needed. Therefore, our objective is to design a prostate cancer immunotherapy strategy that will 1] Enhance the function of tumor antigen-specific T cells by targeted modulation of immune receptor function and 2] Lead to the development of a clinically effective prostate cancer immunotherapy, without inducing severe autoimmunity. We have designed an innovative approach for targeted delivery of antibodies to sites where anti-tumor T cells are induced, using dendritic cell (DCs) transfected with antibody-encoding RNA. When we immunized mice with DCs transfected with tumor antigen RNA and anti-CTLA4 RNA, we observed enhanced anti-tumor immunity, without autoimmunity. Specific to the prostate cancer antigen PAP, we activated more potent anti-PAP cytotoxic T lymphocyte (CTL) responses *in vitro* using DCs modified to express PAP and secrete the anti-CTLA4 antibody. We hypothesize that a vaccine consisting of DCs modified with RNA encoding PAP and anti-CTLA4 will result in increased immunogenicity toward PAP over PAP alone. As described in our proposal, we will conduct preclinical studies with our lead cellular therapy - DCs modified with RNAs encoding PAP and anti-CTLA4 antibody - DC-PAPvac-C. Our intention is to advance this product into clinical trials.

KEYWORDS:

dendritic cell vaccine, dendritic cells electroporated with RNA, immune checkpoint blockade, local CTLA4 modulation, prostate cancer immunotherapy, prostatic acid phosphatase (PAP), and RNA-based vaccines

OVERALL PROJECT SUMMARY:

Protocols specific to this proposal and approval dates:

Animal Use Regulatory Protocols (Aim 1):
Duke University IACUC Approval (Protocol A082-13-03): 03-28-2013 valid for 3 years, annual review approved on 03-26-2015
Title: Novel Immune Modulating Cellular Vaccine for Prostate Cancer Immunotherapy
ACURO Approval (Protocol reference number PC121288): 07-16-2013

Human Use Regulatory Protocols (Aims 2 and 3):
Duke University IRB Approval (Protocol Pro00044351): 04-11-2013 for 1 year
Title: Healthy volunteer leukapheresis for in vitro immune assays
HRPO Approval (HRPO A-17872.2, Proposal Number PC121288, Award Number W81XWH-13-1-0423): 08-15-2013
Duke University IRB Continuing Review Approval: 04-11-2014 for 1 year (approved on 03-14-

2014)
HRPO A-17872.2, Continuing Review Acceptance Memorandum: 04-25-2014
Duke University IRB Continuing Review Approval: 04-11-2015 for 1 year (approved on 03-15-2015)
HRPO A-17872.2, Continuing Review Acceptance Memorandum: 04-21-2015

Duke University IRB DECLARATION OF RESEARCH NOT INVOLVING HUMAN SUBJECTS (Protocol Pro00057900): IRB Declaration is in effect from 09-25-2014 and does not expire.
Title: Tumor infiltration with immune effectors
HRPO Research Not Involving Human Subjects Determination Memorandum (HRPO A-17872.1, Proposal Number PC121288, Award Number W81XWH-13-1-0423): 10-07-2014

Major Task 1: Determine the in vivo systemic and tumor-associated immune response that correlates with anti-tumor activity of the lead cellular therapy, DC-PAPvac-C (months 1-30)
Subtask 1 (1-3 months): Generate mouse PAP RNA, anti-mouse CTLA4 RNA, mouse actin RNA and control IgG RNA for murine immunotherapy studies in TRAMP mice
Status: Completed (Year 1 report)
To evaluate the lead cellular therapy (DC-murinePAPvac-murineC) in a preclinical in vivo setting we will use DCs transfected with RNA encoding mPAP and murine anti-CTLA4, cloned from hybridoma 9H10 as a vaccine in the TRAMP murine model for prostate cancer. Therefore, our first task was to clone the cDNAs of the murine analogs of prostatic acid phosphatase (mPAP) into pSP73-Sph/A64. Since, in our in vitro human studies, hPAP that was cloned without its signal sequence elicited a better CTL response we will test mPAP without the endogenous signal sequence (SS). We will also test mPSMA (murine prostate-specific membrane antigen) without the signal sequence for its potential use as an antigen.
Year 1 report: The following have been cloned and the task was completed within the 3-month timeline and details provided in year 1 report.
Anti-murine CTLA4 RNA
Murine PAP
Murine PSMA
Murine PAP no signal sequence (mPAP-SS)
Murine PSMA no signal sequence (mPSMA-SS)

Year 2 (current) report: In addition to what we proposed to do, we wanted to determine if other prostate associated tumor antigens may be more potent at inducing an anti-prostate cancer immune response. To this end, we decided to look at expression of various prostate tumor antigens in the mouse prostate cell lines and have cloned additional antigens for this study.
Murine PSCA: Attempts to clone full-length murine prostate-specific stem cell antigen (mPSCA) from TRAMP-C1, TRAMP-C2 and murine prostate by reverse transcription-PCR were unsuccessful. A plasmid containing the cDNA for mPSCA was a generous gift of Dr. Owen Witte (UCLA). The coding sequence for mPSCA was amplified from the plasmid using the following primers: forward, 5'-TATATAAGCTTGCCACCATGAAGACAGTCTTCTTTCTC-3' and reverse, 5'-TATATAGGATCCCTACAGACGGCTGGAGCCCCAC-3'. The resulting PCR product was digested with HindIII and BamHI and cloned into the HindIII and BamHI sites

of pSP73-Sph/A64.
Murine STEAP: The cDNA for mSTEAP was obtained from Transomic Technologies. The coding sequence for mSTEAP was amplified by PCR using the following primers: forward, 5'-CGACTCTAGAGGATCCACCATGGAGATCAGT-3' and reverse, 5'-CGGTACCCGGGGATCCCTACAACCTGGAGGCCAT-3'. The resulting PCR product was cloned into pSP73-Sph/A64 digested with BamHI, using the In-Fusion cloning system (Clontech).

Expression of the various antigens in TRAMP-C1 and TRAMP-C2 cells was analyzed and is shown in Figure 1 below. Both cell lines express all antigens being targeted by our DC-RNA vaccines. We are primarily using TRAMP-C1 cells for our subcutaneous mouse tumor model.

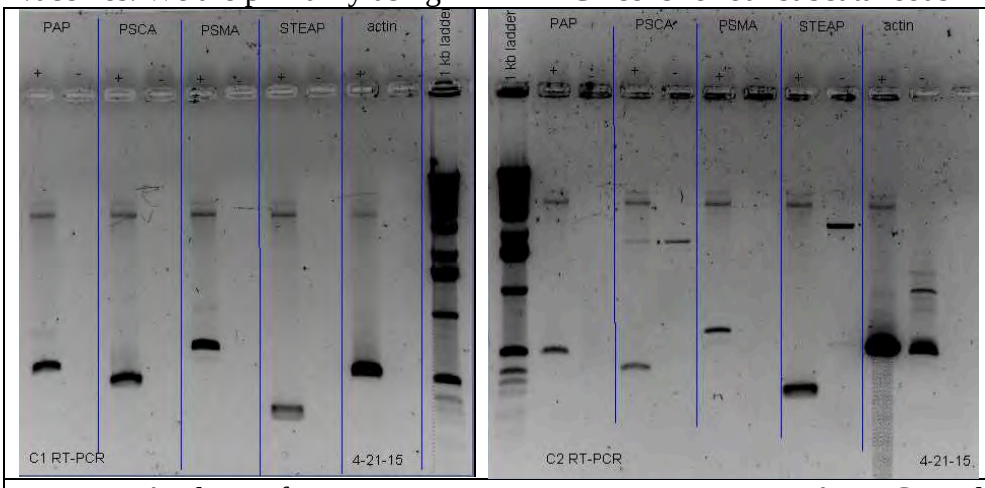


Figure 1. Analysis of prostate tumor antigen expression in TRAMP-C1 and TRAMP-C2 cells. RNA was isolated from TRAMP-C1 and -C2 cells grown in tissue culture. Reverse transcription was primed with a modified oligo dT primer and the following primer pairs were used to amplify potential prostate tumor antigens. Murine β-actin was included as a control:
mPAP: forward, 5'-CTTTCCTACTGACCCCATTAC-3'; reverse, 5'-ATTGTGAACACTCTCGCAG-3', which amplifies a 505 bp sequence of mPAP
mPSCA: forward, 5'-TTCTCCTGCTGGCCACCTAC-3'; reverse, 5'-GCAGCTCATCCCTTCACAAT-3', which amplifies a 403 bp sequence of mPSCA
mPSMA: forward, 5'-GGGAAGATTGTGATTGCCAGAT-3';
reverse, 5'-GCCTCCGTCCTTTCTTCTTCA-3', which amplifies a 643 bp sequence of mPSMA
mSTEAP: forward, 5'- GGTGGCTGAAGCCGTACTAT-3';
reverse 5'- GGATGATATGATGGCAGCGAC-3', which amplifies a 281 bp sequence of mSTEAP
mActin: forward, 5'- ATGGTGGGAATGGGTCAGAAGGAC-3';
reverse, 5'- CTCTTTGATGTCACGCACGATTTC-3', which amplifies a 513 bp sequence of mβ-actin
PCR products were loaded onto a 1% agarose gel and analyzed. Left panel: TRAMP-C1 cells; Right panel: TRAMP-C2 cells

Subtask 2 (3-6 months): Start first experiment with TRAMP mice, immunize mice using DC-mPAPvac-mC and controls
Status: Completed (Year 1 report)

Subtask 3 (6-12 months): Analyze immune responses in the periphery: T cell analysis, ELISpot analysis anti-PAP antibody analysis
Status: Completed (Year 1 report)

Subtask 4 (8-20 months): Harvest tumors from 30-week old mice to analyze tumor weight, tumor grade, tumor apoptosis and immune infiltrates and harvest mouse organs (lymph nodes, lungs, kidney, testis, colon, liver, muscle) for analysis of inflammatory infiltrates and autoimmunity
Status: Ongoing
As proposed in our specific aims, we will also determine vaccine-induced immune infiltrate changes in mouse prostate and prostate tumors. We have optimized staining of these tissues by immunofluorescence in the TRAMP-C1 subcutaneous tumor model and this analysis is ongoing. Preliminary analysis demonstrates T cell infiltration in subcutaneous TRAMP-C1 tumors (Figure 2). Analysis of tumor grade and tumor weight in TRAMP transgenic mice is pending.

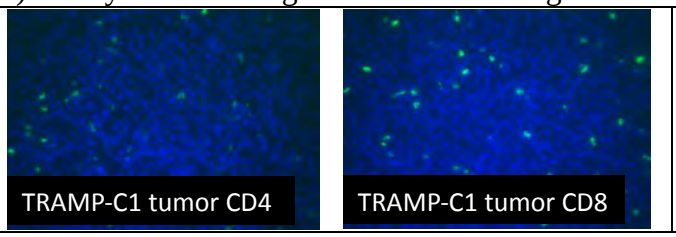


Figure 2. T cell infiltration in subcutaneously implanted TRAMP-C1 prostate tumor cells. 7 μm sections of OCT-embedded TRAMP-C1 tumors were stained with DAPI (blue, nuclei) and FITC-labeled anti-CD4 or anti-CD8 plus (green).

Subtask 5 (12-15 months): Start second experiment with TRAMP mice, immunize mice using DC-mPAPvac-mC and controls
Status: Completed
We are now using the TRAMP transgenic mouse model and the TRAMP-C1 subcutaneous tumor model. Descriptions are provided in the next section.

Subtask 6 (15-21 months): Analyze immune responses in the periphery: T cell analysis, ELISpot analysis and anti-PAP antibody analysis
Status: Completed
TRAMP transgenic mouse model: TRAMP male mice are vaccinated 3 times at weekly intervals with 4 x 10^5 DCs transfected with mPAP-SS mRNA or with mPAP-SS mRNA plus anti-CTLA4 mRNA. Vaccination starts when mice are 10-12 weeks old. Mice are sacrificed 7-10 days after the last immunization for analysis of T cell and antibody responses
TRAMP-C1 subcutaneous tumor model: TRAMP-C1 cells were established from a tumor harvested from a 32-week-old C57BL/6 TRAMP male and are tumorigenic following subcutaneous injection into the nontransgenic C57BL/6 male mouse. We can assess various parameters in this model due to the quicker time to tumor onset post-subcutaneous tumor implantation. We are using this model to determine efficacy of our vaccine strategy in parallel to using the TRAMP transgenic mice. Mice are vaccinated 2 times at weekly intervals with 4 x 10^5 DCs transfected with mPAP-SS mRNA or with mPAP-SS mRNA plus anti-CTLA4 mRNA. Mice are implanted with tumor cells on day 0 followed by vaccinations on days 7 and 14. Mice are sacrificed 7-10 days after the last immunization for analysis of T cell and antibody responses.
IFN-γ ELISpot assay: Untouched CD4+ T cells and CD8+ T cells are isolated from spleens using magnet-bead based techniques (Miltenyi). 50,000 T cells per well of a 96 well Multiscreen-IP plate (Millipore) are stimulated at a stimulator to effector ratio of 1:10 as indicated. In addition to

TRAMP-C1 cells and B16-F10 melanoma cells as control, additional stimulators are included as indicated in the figures. Plates are incubated for 24 hours at 37°C and plates are developed using an anti-mouse IFN-γ ELISpot kit (BD Biosciences) per kit instructions. Results are presented as spots per 10^5 CD8 or CD4 T cells. Stimulator tumor cells used for ELISpot analysis are shown in Figure 3.

Anti-PAP antibody responses: A standard ELISA assay is performed by coating ELISA plate with recombinant PAP protein. Serum from immunized mice is analyzed and compared to commercially available anti-PAP monoclonal antibody (mAb) as a positive control.

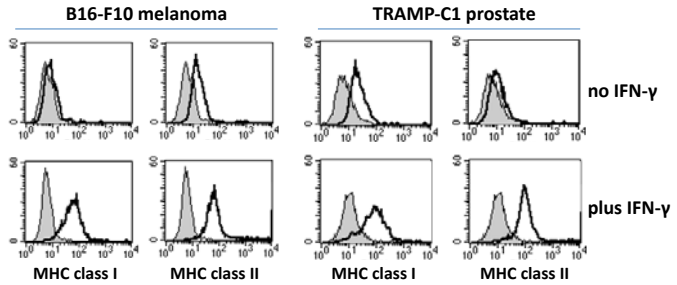


Figure 3. Stimulator cells used for CD8 and CD4 T cell analysis by ELISpot. Flow cytometry analysis of B16-F10 melanoma cells and TRAMP-C1 cells pre- and post-treatment with IFN-γ. Both class I and class II MHC molecules are upregulated post-IFN-γ making them suitable stimulators for analysis of prostate antigen-specific CD8 and CD4 T cells.

In Figure 4 we demonstrate the induction of anti-PAP antibody responses in vaccinated mice in the TRAMP transgenic mouse model and the TRAMP-C1 subcutaneous mouse model. As expected, DC-mPAP-SS RNA induced lower levels of antibody, because mPAP-SS is engineered without a signal sequence and is therefore not secreted. Although we observed an anti-PAP antibody response in the TRAMP transgenic mouse model, we were not able to detect anti-PAP induction in the TRAMP-C1 subcutaneous (subcu) model.

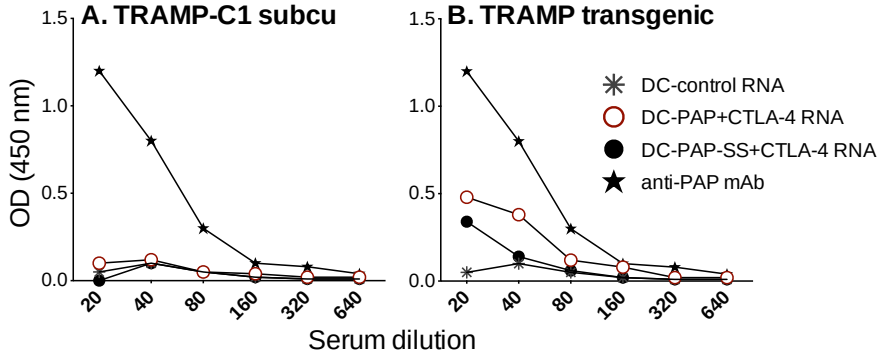


Figure 4. Analysis of anti-PAP antibody response in immunized mice. Mice were immunized as indicated and serum analyzed for anti-PAP antibody. Panels A and B represent the 2 models used for this analysis.
subcu, subcutaneous

In Figure 5 we demonstrate the induction of anti-PAP CD4 and CD8 T cell responses in vaccinated mice. Mice were vaccinated as indicated above for the TRAMP transgenic model and the TRAMP-C1 subcutaneous model. Mice are sacrificed 7-10 days after the last immunization for analysis of T cell responses. Results are presented as spots per 10^5 CD8 or CD4 T cells. As expected, frequencies of PAP-specific CD4+ T cell responses were low, given that we used PAP that lacks a signal sequence that has limited access to MHC class II presentation. Clearly, the mPAP-SS + anti-CTLA4 DC vaccine was superior to PAP + anti-CTLA4 DC vaccine in the induction of CD8 T cell responses, which likely due to lack of the signal sequence and the retention of the antigen in the cytoplasm.

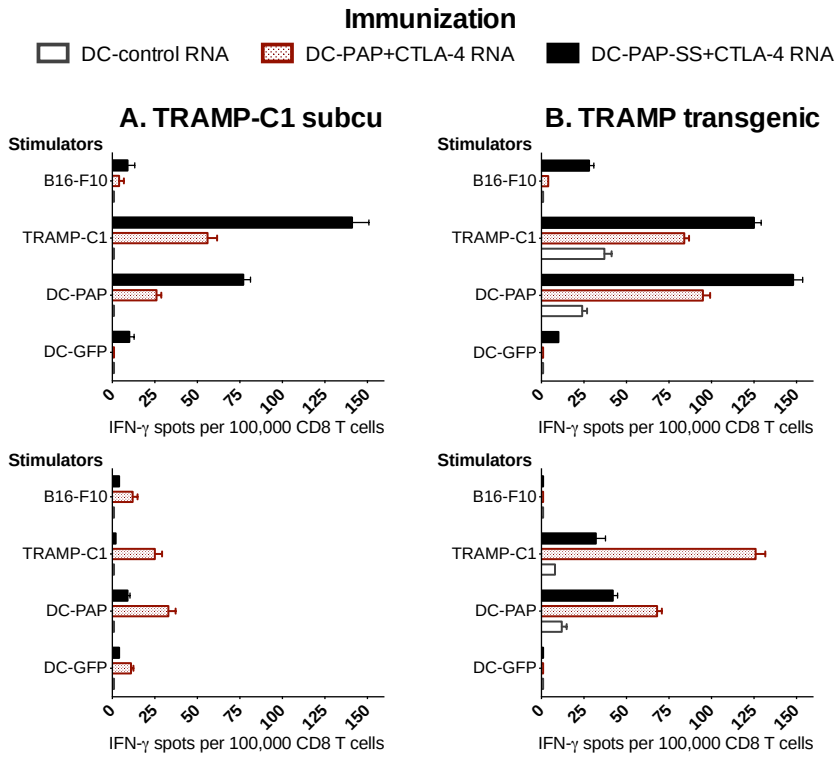


Figure 5. Analysis of PAP-specific CD8 and CD4 T cells by IFN-γ ELISpot. Mice were immunized as indicated and splenocytes were harvested and untouched CD4 and CD8 T cells were isolated prior to analysis of PAP-specific T cells. ELISpot analysis procedure is described above. B16-F10 and TRAMP-C1 cells were used for this analysis based on class I and class II MHC expression data in Figure 3 above. In addition, we used DC stimulators transfected with PAP RNA (positive control) and GFP RNA (negative control) to analyze antigen specificity of the T cells response. Panels A and B represent the 2 models used for this analysis.

In Figure 1 we demonstrated the expression of PAP, PSCA, PSMA and STEAP in the prostate cell lines TRAMP-C1 and TRAMP-C2. Both are cell lines that have been generated by in vitro propagation of tumors isolated from TRAMP transgenic mice. One of the main reasons for examining other prostate antigens was to determine if we could generate a vaccine that targets all of these antigens, instead of PAP antigen alone. So in the next experiment, we immunized TRAMP transgenic mice as described above with DCs transfected with all the prostate tumor antigen RNAs. Instead of co-transfecting a single DC prep with all four RNAs, we transfected DCs with each RNA and then combined the DC-RNA preparations prior to immunization. Therefore our DC vaccine consisted of a combination of 4 different DC formulations with 200,000 cells of each of the following: DC-PAP + DC-PSCA + DC-PSMA + DC-STEAP to give a combined dose of $8x10^5$ DCs per vaccination. In Figure 6 we demonstrate that we are indeed able to generate antigen-specific CD4 and CD8 T cells that target each of the antigens used. RNA-transfected DCs were used as ELISpot stimulators, along with TRAMP-C1 cells and control B16-F10 melanoma cells. Among the different antigens tested, PAP specific responses seem to be lower than the responses induced against the other prostate tumor antigens.

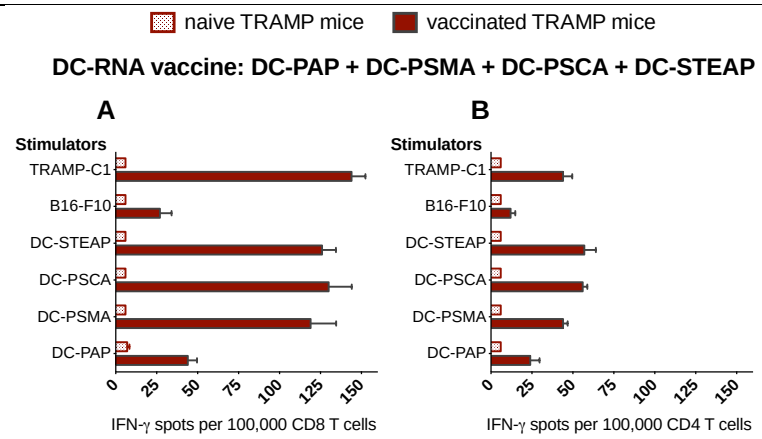


Figure 6. Analysis of prostate tumor antigen specific CD8 and CD4 T cell responses in TRAMP transgenic mice immunized with DC-RNA vaccine. Untouched CD8 and CD4 T cells were analyzed after the third vaccination for induction of antigen specific T cells. T cells from naïve TRAMP mice were used as controls. A, CD8 T cells and B, CD4 T cells.

Subtask 7 (17-30 months): Monitor tumor burden (time to palpable tumor) and monitor survival. If mice have tumors then sacrifice and harvest prostate complex/tumor and analyze as described in Subtask 4

Status: Ongoing

Preliminary data in the TRAMP-C1 subcutaneous tumor model is shown below (Figure 7). The mPAP-SS RNA and CTLA4 RNA transfected DCs seem to provide a therapeutic benefit that is superior to PAP RNA and CTLA4 similar to our observations with human PAP-SS RNA and PAP RNA comparison in the human in vitro CTL assays (preliminary data in grant).

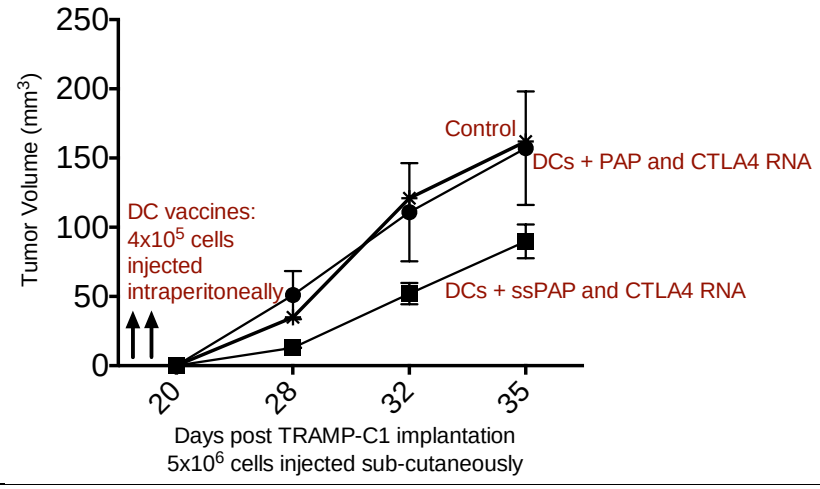


Figure 7. DC-PAPvac-C immunization in the TRAMP-C1 tumor immunotherapy model provides therapeutic benefit. Mice were implanted with tumor cells on day 0 followed by vaccinations on days 7 and 14. Tumor growth was evaluated and is depicted as tumor volume in the figure.

Major Task 2: Using human prostate cancer tissue, determine the presence of immunologic markers that were identified in Aim 1 as correlated with vaccine efficacy. Develop assays to be used in subsequent human clinical trials of DC-PAPvac-C as markers of an effective immune response (months 6-30)

Subtask 1 (6-30 months): Obtain pre- and post-vaccine treatment human prostate tissue and embed them in paraffin and generate sections for immunohistochemistry.

Status: Ongoing

We now have a protocol that has been determined not human subjects research that has been approved by both Duke and HRPO.

To date we have obtained human prostate tissue from 3 patients. The plan is to analyze 20 samples. We will not determine if data are from pre-or post-vaccination tissues until all 20

samples are analyzed. Prior to staining human prostate tissue we standardized staining of CD8 and CD4 T cells using human tonsil tissue (Figure 8). Because we have access to frozen tumor tissue, we will perform immunofluoresence analysis instead of immunohistochemistry. This allows us to avoid the problems associated with tissue processing and antigen retrieval when using paraffin-embedded tissue samples.

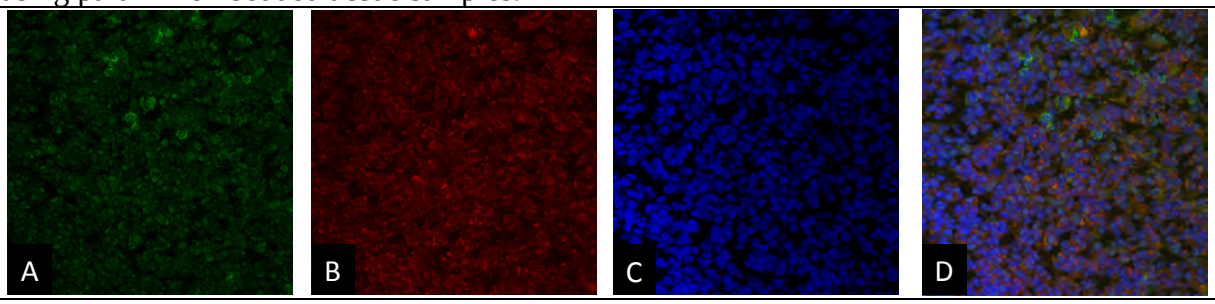


Figure 8. 3-color immunofluorescence in human tonsil. anti-CD8-Green (A); anti-CD4-Red (B) ; DAPI-Blue (C); D, merged image

Subtask 2 (8-30 months): Analyze immune infiltrates
Status: Ongoing

Below we present analysis of CD8 T cells that express Ki67 (proliferating marker) in prostate tumor tissue (Figure 9). As is evident, there was no evidence of CD8 T cells in Patient 2. DAPI is used to demonstrate nuclear staining. CD8 and CD4 T cell and regulatory T cell analysis in human prostate tumor tissue will continue over the period of the grant.

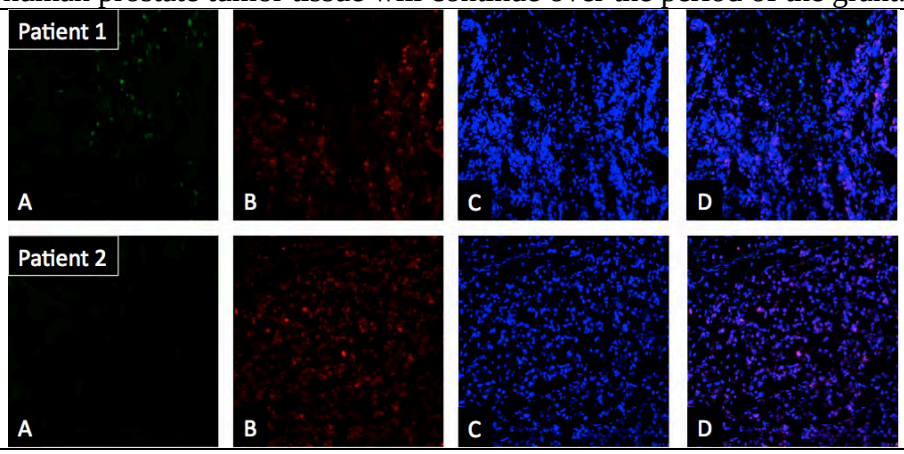


Figure 9. Immunofluorescence analysis of prostate tumor tissue for infiltrating CD8+Ki67+ T cells. Staining was performed on frozen samples. A, Green, anti-CD8; B, Red, anti-Ki67; C, Blue, DAPI. D, merged image.

Major Task 3: Perform FDA mandated validation of DC-PAPvac-C to confirm anti-CTLA4 mAb expression and PAP presentation by human DCs transfected with mRNA encoding hPAP and anti-human CTLA4 (months 1-30)

Subtask 1 (1-9 months): Characterize and optimize human PAP (hPAP) expression and anti-CTLA4 secretion by human dendritic cells (DCs)
Status: Completed (Year 1 report)

Subtask 2 (10-15 months): Evaluate the function of optimized DC-PAPvac-C
Status: Completed (Year 1 report)

Subtask 3 (8-12 months): Generate, validate, test and vial clinical-grade RNA for cGMP

production of DC-PAPvac-C

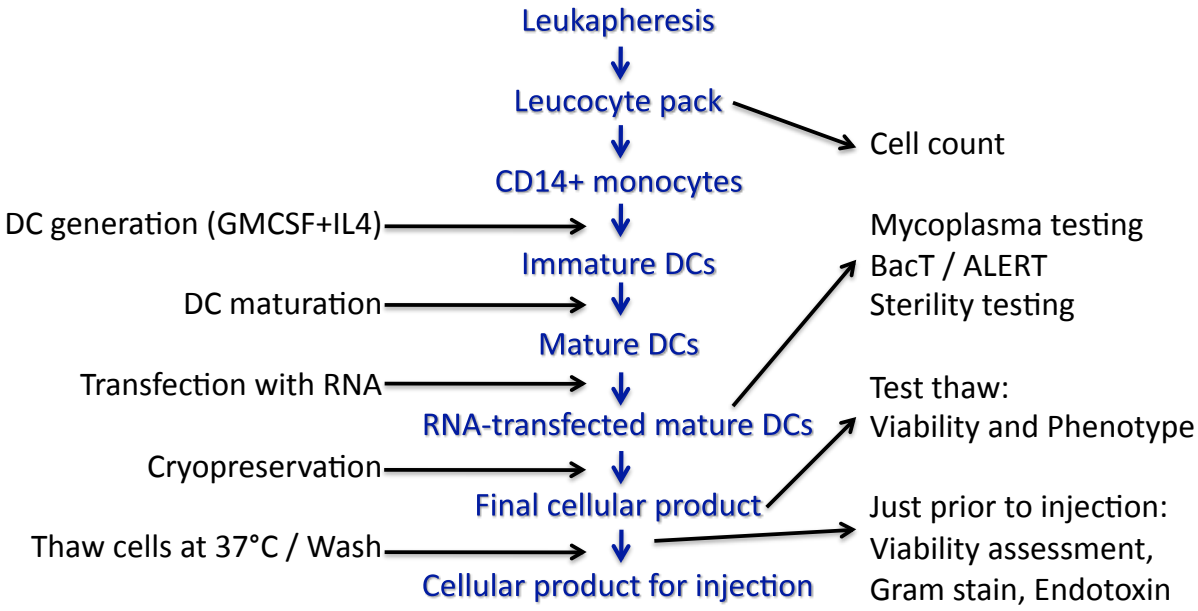
Status: Completed (Year 1 report)

We have generated and vialed cGMP RNA and validated that the RNA is functional. All cell culture regents, cytokines, and mRNA in vitro transcription kits that are used in all qualitative and quantitative analysis is compatible with GMP manufacturing of cellular vaccines.

Subtask 4 (12-30 months): Perform three cGMP production runs including lot release testing of the final DC-PAPvac-C cellular vaccine product in the GMP-certified cellular processing laboratory

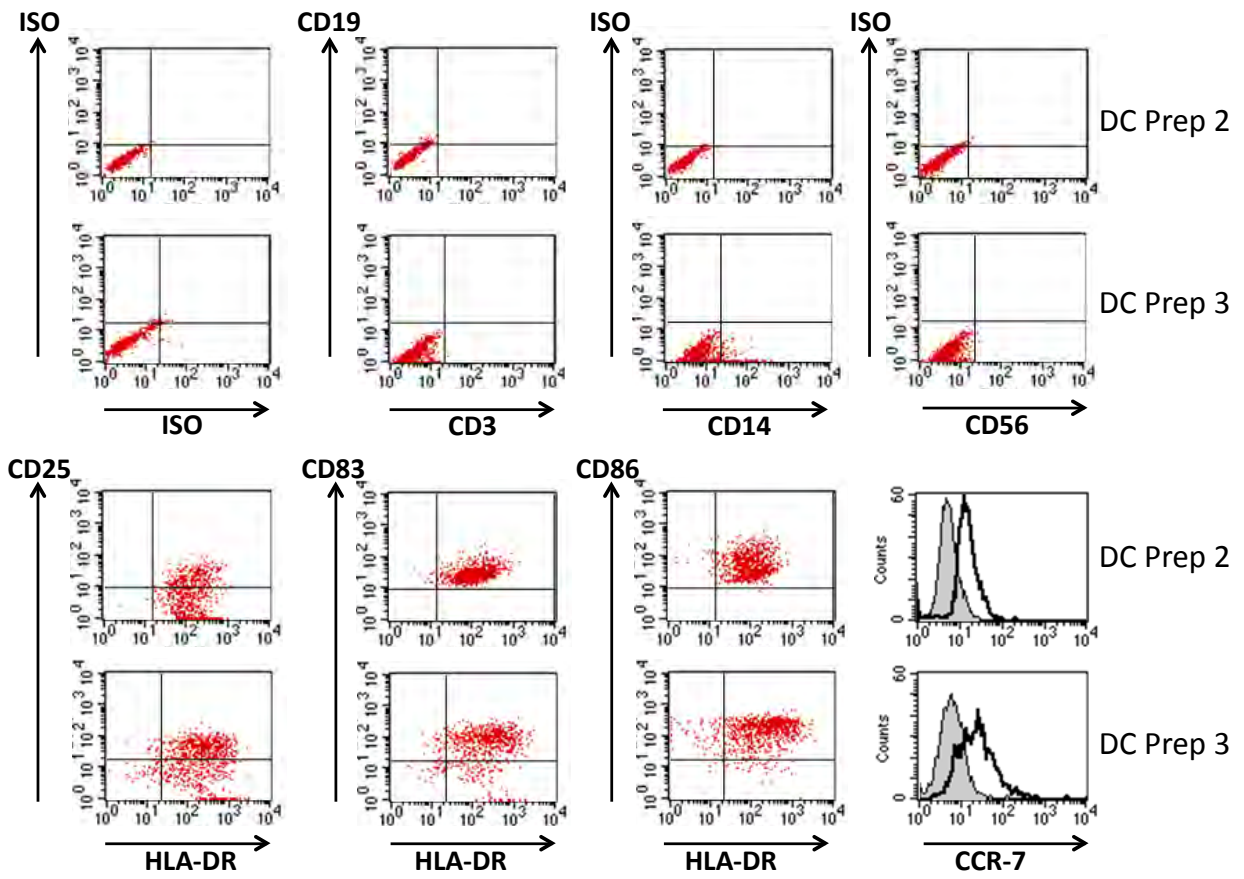
Status: Completed (Year 2 report and Year 1 report)

Quality assurance (QA) and quality control (QC) analysis of DC cellular product, DC-PAPvac-C, (DC prep 2 and 3) and was done using the schematic shown below.



We generated 2 additional DC vaccine preparations under cGMP conditions (DC Prep 2 and DC prep 3). DCs were grown in serum and antibiotic-free media using carrier-free cytokines and immature DCs were harvested. After transfection with mRNAs (10 μg H chain mRNA, 5 μg L chain mRNA, and 5 μg PAP-SS mRNA (all Cap1)), DCs were matured in the presence of TNF-α, IL-1β, IL-6, and PGE2. First, we analyzed if these cells met the phenotypic requirements for batch release. Immature and mature cells were stained with lineage markers CD3 (T cells), CD14 (monocytes/macrophages), CD19 (B cells), and CD56 (NK cells). As can be seen in Figure 10 (top), all lineage markers were expressed by less than 10% of cells as mandated in our previous INDs. Furthermore, DCs acquired >50% expression of maturation markers CD25, CD83, CD86, and HLA-DR upon maturation as required for batch release (Figure 10). Last, DCs up-regulated expression of CCR-7 during maturation which is very important for the migratory properties toward the lymph node-derived chemokine CCL-21 (MIP-3β).

We also tested our cell product for bacterial contamination and the absence of Mycoplasma using the PCR-based MycoSensor test (Agilent Technologies). As can be seen in Figures 11 and 12, our DC preparation was free of bacterial and Mycoplasma contamination.



DC Release Criteria	DC Prep 2	DC Prep 3
CD3 ≤ 10%	0	0.1
CD14 ≤ 15%	0	10.8
CD19 ≤ 10%	3.4	0
CD56 ≤ 10%	0	0.4
CD25 ≥ 40%	58.4	78.3
CD86 ≥ 50%	99.8	94.2
CD83 ≥ 50%	99.7	86.0
HLA-DR ≥ 80%	99.7	88.1
Viability ≥ 80%	90	92
Gram Stain = negative	Negative	Negative
Endotoxin ≤ 5 E.U./kg	Negative	Negative

Figure 10. Phenotype of human monocyte-derived DC preparations 2 and 3. (Top) mRNA-transfected and matured DCs were analyzed for expression of lineage markers and maturation markers. (Top, Bottom row, Left) DCs up-regulate expression of CCR-7 upon maturation. Grey histograms, DCs stained with an isotype control antibody and solid line, DCs stained with anti-human CCR-7. (Left) Table depicting release criteria for DC vaccines. DC preps 2 and 3 fulfill the release criteria for this cellular vaccine product.

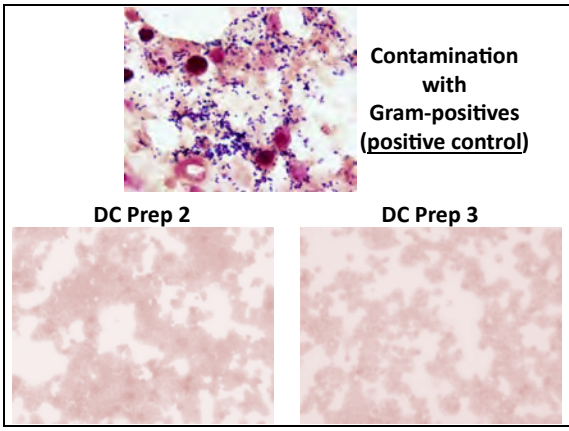
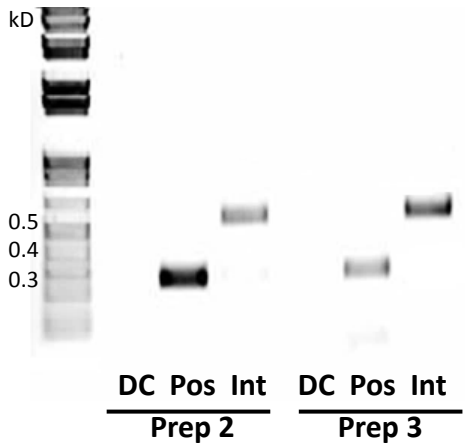


Figure 11. Gram stain analysis to detect bacterial contamination of thawed RNA-transfected mature DC product, DC-PAPvac-C. Frozen DC cellular product was thawed at 37°C and washed. 10,000 DCs were cytospun and analyzed using standard bacterial gram stain protocol. Slides were analyzed at 1000x.

Figure 12. MycoSensor PCR for detection of Mycoplasma contamination in DC-PAPvac-C. DNA was isolated from 100,000 DCs and 100 cell



equivalents were analyzed by PCR according to the manual provided by the manufacturer. A DNA band at 0.32 kD indicates Mycoplasma contamination. An internal control band at 0.52 kD indicates that sample preparations do not inhibit the PCR reaction. Pos, PCR reaction with added Mycoplasma DNA; Int, PCR reaction with added control DNA; DC, PCR reaction in the presence of DNA isolated from DC preparations 2 and 3. Absence of a 0.32 kD band in PCR reactions containing DNA isolated from DCs (DC) indicates the absence of Mycoplasma contamination.

KEY RESEARCH ACCOMPLISHMENTS:

1. In murine studies, we have demonstrated that local CTLA4 modulation in combination with PAP-specific immunization using DCs transfected with PAP RNA and CTLA4 RNA is superior to DC PAP RNA immunization in T cell function assays and in the TRAMP-C1 subcutaneous tumor model.
2. In murine studies, we have demonstrated that DCs transfected with mPAP-SS RNA and CTLA4 RNA is superior to DC PAP RNA and CTLA4 RNA immunization in T cell function assays and in the TRAMP-C1 subcutaneous tumor model. We have also demonstrated the induction of anti-PAP antibodies in immunized mice.
3. We have conducted experiments to evaluate prostate tumor antigen expression in TRAMP-derived cell lines. Based on our data we have cloned additional antigens, PSCA and STEAP, for testing in DC-RNA vaccines. We show that among the 4 antigens used as vaccines in TRAMP transgenic mice, anti-PAP T cell response was the weakest as compared to T cell responses directed towards PSMA, PSCA and STEAP.
4. Using human cells, we have performed FDA mandated validation of DC-PAPvac-C to confirm anti-CTLA4 mAb expression and PAP presentation by human DCs transfected with mRNA encoding hPAP and anti-human CTLA4.
5. We have completed the three cGMP production runs including lot release testing of the final DC-PAPvac-C cellular vaccine product.

CONCLUSION:

Recently, two forms of immunotherapy have demonstrated clinical benefit in patients: active immunotherapy, in which subjects are immunized with antigen presenting cells activated against tumor antigens ex vivo (e.g. sipuleucel-T in prostate cancer) and treatment with systemic immune modulators, such as an antagonistic anti-CTLA4 mAb (e.g. ipilimumab in melanoma). However the use of anti-CTLA4 was associated with adverse events. We have developed a novel strategy that combines tumor immunotherapy targeting PAP and targeted immune modulation of CTLA4 and have generated a lead cellular therapy that will safely enhance vaccine-mediated immunity. This lead cellular therapy, called DC-PAPvac-C, consists of autologous monocyte-derived DCs co-transfected with prostate tumor antigen, PAP RNA and anti-CTLA4 RNA. Thus, targeted delivery of anti-CTLA4 antibody to sites where anti-tumor T cells are induced by tumor antigen-presenting DCs will potentially eliminate adverse effects associated with systemic administration of anti-CTLA4, while also enhancing vaccine-induced immune responses and

expanding the potential role for immunotherapy in patients with cancer. In this study we will establish the preclinical efficacy and safety of our cellular therapy product, DCs transfected with RNA that encodes PAP and anti-CTLA4 and generate data required for an IND application. Importantly and relevant to our planned clinical trial implementation, we will develop a biomarker of therapeutic efficacy and demonstrate the feasibility of measuring these biomarkers.
In this report we have demonstrated that local CTLA4 modulation in combination with PAP-specific immunization using RNA-transfected DCs elicits robust and superior functional T cell responses in TRAMP mice. Using human cells, we have performed FDA mandated validation of DC-PAPvac-C to confirm anti-CTLA4 mAb expression and PAP presentation by human DCs transfected with mRNA encoding hPAP and anti-human CTLA4. Finally, we have completed the three cGMP production runs including lot release testing of the final DC-PAPvac-C cellular vaccine product.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:
Nothing to report

INVENTIONS, PATENTS AND LICENSES:
Nothing to report

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Nothing to report

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Nothing to report

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None

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None